International Journal of Zoology Studies
ISSN: 2455-7269; Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 1; Issue 5; July 2016; Page No. 45-49

A review on attributes of Vitamin C with particular reference to the silkworm, *Bombyx Mori* Linn

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Abstract

Vitamin C (VC) is a water soluble derivative of sugar molecule which includes all the compounds having antiscorbutic activity. This multifunctional compound which melts at 190-192°C with decomposition is a strong monobasic acid widely distributed in nature, particularly in the plant kingdom. Most insects including silkworm are unable to synthesize the substance. The animals which cannot synthesize VC, lack an enzyme, L-gulono-1, 4-lactone oxidase, which is essential for synthesis of the VC immediate precursor 2-keto-l-gulonolactone. All plant feeding insects including silkworm are reported to need VC. VC is known to serve as phagostimulant and growth promoter for phytophagous insects and its deprivation in diet results in impaired development. In silkworm, dietary fortification of mulberry leaves with VC has been reported to increase the food consumption, coefficient of utilization and economic traits larval weight within optimum limits. The excessive amounts of VC supplementation to silkworm diet have negative impact causing decrease in food intake and cocoon characteristics due to hypervitaminosis.

Keywords: vitamin C, ascorbic acid, silkworm, insects

1. Introduction

The term "vitamin" is derived from the Latin word 'vita' meaning life and describes a class of nutrients that regulate important metabolic reactions in the body (Lieberman and Bruning, 1990) [47]. Funk (1912) [32] referred vitamins as "vital amines". The 'e' was later dropped and the compound class is now universally called as vitamins (Davies *et al.*, 1991) [21]. Their function is to promote a wide variety of biochemical and physiological processes necessary for life. Collectively, vitamins assist in the formation of a wide spectrum of biochemicals including hormones, enzymes, proteins, neurotransmitters, and the genetic materials RNA and DNA. Vitamins are divided into two categories, one of which is commonly known as fat soluble and includes vitamins A, D, E, and K. The other, water soluble, vitamin category includes the B-vitamin Complex and vitamin C.

Vitamin C is chemically the simplest of the vitamins and perhaps for these reasons it was first isolated, characterized and its structure determined. It is a nutrient required in very small amount to allow a range of essential metabolic reactions in the body. It is principally a water-soluble anti-oxidant known by the chemical name of its principal form L-ascorbic acid or simply ascorbic acid or ascorbate (abbreviated as VC here).

1.1 Historical perspective of vitamin C

The name "ascorbic acid" comes from *Greek root*-"a" (no or anti) and "scorbutus" (Scurvy) or simply "anti-scurvy" acid; because it was known to dramatically cure this disease. This disease was caused by a serious deficiency of VC, rendering its victim's small blood vessels to rupture, bones to weaken and joints to swell, among other symptoms (Sauberlich, 2000) [64]. Lind (1753) [48], a Scottish naval surgeon, to find out the cause for scurvy conducted controlled experiments on sailor's diets and published his findings as "*Treatise on the Scurvy"* and as a result, in 1795 daily doses of lime juice were prescribed to all the sailors in the British navy and scurvy quickly vanished. However, the British were the only people who accepted the idea that scurvy was the result of a dietary deficiency. These findings were not widely accepted by rest of the world throughout the 19th century (Groff *et al*,. 1995) [35]. Drummond (1919) [28] described antiscorbutic factor as "Water soluble C". As the term 'vitamin' was adopted for essential factors, so the antiscorbutic factor was designated as vitamin C (Bucci, 1998) [14]. In 1928, Szent-Gyorgyi, working on a biochemical problem unrelated to scurvy or VC, reported that he has isolated crystals of a new sugar-like substance with very unusual chemical properties from the adrenal gland of the ox. He first named it "ignose" then "godnose" and finally "hexuronic acid" which gave colour tests characteristic of sugars (Coultate, 2002) [20]. Similar crystals were isolated from oranges and cabbages. While these crystals were isolated in connection with another biochemical problem, Szent-Gyorgyi noted their similarity to the chemical reaction of VC and suspected there was some connection. He made arrangements to have the crystals tested by animal assay which proved hexuronic acid to be vitamin C.

1.2 Structure and properties of vitamin C

Vitamin C ($C_6H_6O_8$) is the generic descriptor for compounds having antiscorbutic activity (Plate 1). These include salts and esters of ascorbic acid and its oxidative derivative L-dehydroascorbic acid. The chemical name is 2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol (Moser & Bendich, 1990). Depending on the nomenclature used it has a number of formal systematic names, for example, L-3-ketothreohexuronic acid-γ-lactone (Belitz and Grosch, 1999) [13]. VC is the enolic form of 3-oxo-L-gulofuranolactone. VC is a hexose derivative and is nominally a carbohydrate. Acidic carbohydrates are generally thought of as containing carboxylic acid group, but no free carboxylic acid group is present in VC. A carboxylic acid group has been lost to the lactone structure. Rather, the hydroxyl groups on carbon 2 and 3 are ionisable. VC is very sensitive to even slight heating, light, and action of oxidizing agents and metal ions. VC is readily oxidized, especially in aqueous solutions, by reacting with atmospheric oxygen, and behaves as

a two-electron donor. The oxidation of ascorbic acid to dehydroascorbic acid (DHA) is freely reversible and the further reaction of the later leads to an irreversible hydrolysis of its lactone yielding 2, 3-keto-1-gulonic acid (DKG).
It is a white crystalline optically active solid which melts at 190-192°C with decomposition, and is a strong monobasic acid. Redox potential of first stage at pH 5.0 is E 0 = + 0.127 V. In aqueous acid solution (pH 2), it exhibits an absorption maximum at 243 nm. At pH 6 to 10, the maximum lies at 265 nm and in more strongly alkaline solutions the maximum occurs at 294 nm. VC is highly polar compound, which is highly soluble in water at ambient temperature (330 g/L) (Eittenmiller and Landen, 1999) [29] but insoluble in non-polar solvents
Though it is known by many scientists, it is virtually unknown by the public that VC has two totally and distinctly different separate sides. The two sides of VC consist of L-ascorbic acid which is the (-) side and D-ascorbic acid which is the (+) side. It is the L-ascorbic acid that is the active side and the side that is beneficial to mankind. Most research shows that the D-side of ascorbic acid is discarded by the body and designated as little or no-use. People ingesting VC are therefore only likely to benefit from the L side of VC (Mason: www.antiaging-systems.com). Vitamin C in foods occurs in complex with bioflavonoids, amino acids, fiber etc. known as food VC complex.

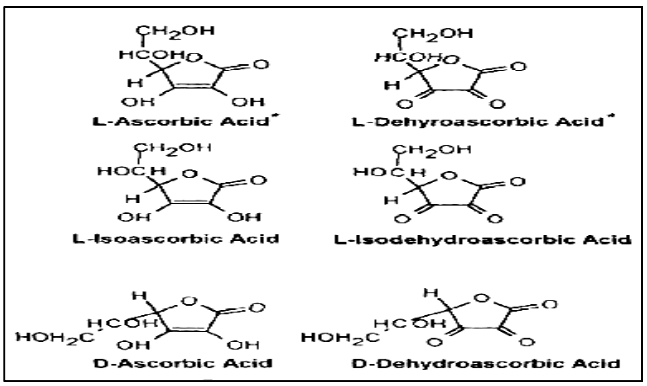

Plate 1: Structure of vitamin C and related compounds. The two compounds marked with asterisk have vitamin C activity. Isoascorbic acid (stereoisomer) has little scorbutic activity (Gregory, 1996).

1.3 Vitamin C synthesis in insects and other animals
Vitamin C is synthesized *de novo* by a number of insects and most insects are unable to synthesize adequate amounts of the substance from glucose. Although the biosynthesis of VC in insects has not been studied thoroughly, the pathway appears to be similar to that found in vertebrates. VC is manufactured from glucose by nearly all the animals. The conversion proceeds stepwise, each step being controlled by a different enzyme. The animals which cannot synthesize VC, lack an enzyme, L-gulono-1, 4-lactone oxidase, which is essential for synthesis of the VC immediate precursor 2-keto-l-gulonolactone. Of special interest is the finding that the intermediates from the pathway of VC synthesis in vertebrates (d-glucuronic acid, D-glucuronolactone and l-gulonolactone) do not substitute for VC in those insects which require the vitamin. Therefore, these insects also may be deficient in L-gulonolactone oxidase. The silkworm *Bombyx mori* L. also belong to the group of insects which do not synthesize the VC (Ito and Arai 1965) [40]. The DNA encoding for gulonolactone oxidase is reported to have undergone substantial mutation, resulting in the absence of the functional enzyme (Sato & Undenfriend, 1978 [63] and Nishikimi and Yagi, 1996) [58].
In animals, VC is synthesized in the liver or kidneys by conversion of D-Glc as part of the hexuronic acid pathway (Nishikimi and Yagi, 1996 [58] and Banhegyi *et al.,* 1997) [11]. VC is synthesized in animals along the following route.

D-Glucose → D-glucurono-y-lactone→ L-gulono-y-l’

Some fungi can synthesize erythroascorbic acid, a VC analogue with similar metabolic functions. Among prokaryotes, only cyanobacteria have been reported to have a small VC amount (Arrigoni and De Tullio, 2002) [9].

1.4 Importance in insects
Vanderzant and Richardson (1963) [70] reported that all the insects known to need VC are plant feeders. VC is known to serve as phagostimulant for phytophagous insects (Cohen, 2004) [19]. On the other hand, VC was shown to elicit no significant feeding response for several sugar-cane infesting scarabaeids *Antitrogus parvulus* and *Lepidiota negatoria* (Allsopp, 1992) [22]. Dadd (1957 & 1960) [25], Vanderzant *et al.*, (1962) [71], Chippendale & Beck (1964) and Levinson & Navon (1969**)** regarded dietary VC essential for the growth and development of several plant-feeding insects and its omission from diet caused retarded growth and heaviest mortality at the moult from the fourth to the fifth instar. VC has been reported to be associated with larval moulting (cuticular sclerotization) and development of mouth parts in insects (Navon, 1978 & Chapman, 1998) [57, 16]. VC could also control the hydroxylations that convert cholesterol to the insect molting hormone, ecdysone (Rockstein, 1978) [62]. VC could regulate the enzymatic hydroxylation of tyrosine to dihydroxyphenylalanine, which is the first step in the formation of the tanning agent, N-acetyldopamine (Dadd, 1960 [25] & Sekeris, 1972) [66]. Felton and Duffey (1992) [31] provided evidence of an important role for ascorbate free radical (AFR) reductase, dehydroascorbate (DHA) reductase, glutathione and glutathione reductase as components of an oxidant-scavenging system in the midgut of larval *Helicoverpa zea*. Barbehenn *et al.*, (2001) [12] demonstrated that an ascorbate-recycling system in the midgut lumen can act as an effective antioxidant defense in *Malacosoma disstria* and *Orgyia leucostigma* caterpillars that feed on prooxidant-rich food. Mathews *et al.*, (1997) [51] demonstrated that ascorbate peroxidase (APOX) activity, which catalyzes the oxidation of VC with the concurrent reduction of hydrogen peroxide (H_2O_2), was important in removing lipid peroxides in fat body tissues of *Helicoverpa zea* larvae.

1.5 Importance in silkworm
The silkworm, *B. mori* has been classified among the insects which are unable to synthesize VC in their body and depend on exogenous supply to fulfill the requirement (Ito and Arai 1965) [40]. Neither D-glucunolactone nor D-gulonolactone replaced VC and it supported the evolutionary theory on the absence of biosynthetic pathway of VC in the silkworm, *B. mori* (Ito, 1978). Several researchers have demonstrated phagostimulatory effect of VC (Ito, 1961a, 1961b, 1978 [38], Ito & Arai, 1965 [40] and Dobzhenok, 1974). During the first instar, a stimulatory effect of VC on silkworm voluntary feeding was postulated by Ito (1961a) [38]. in silkworm a gustatory stimulating activity have been observed to some extent (Ito, 1961b) [38]. It was

demonstrated by an electrophysiological method that the sensilla styloconica SS-I and SS-II on the maxillary tubercle of the silkworm are sensitive to VC (Cui *et al.*, 2001) [23]. The availability of artificial diet from the 1960s, permitted researchers to confirm the importance of VC for the silkworm physiology on the basis of classical experiments carried out by Ito, though it is difficult to precisely estimate the amount of VC ingested by silkworm larvae fed fresh mulberry leaves. VC usually has been added to silkworm food (enrichment) in a quantity generally varying from 1–2% of the dry weight of the artificial diet, which is considered as optimum content of this vitamin (Ito, 1978) [39]. Vitamin C has always been regarded as indispensable for the growth and development of the silkworm, *Bombyx mori* and its deprivation in the diet affected larval growth and cocoon production (Ito, 1961 [38] & Cappellozza *et al.*, 2005) [15]. Cui *et al.*, (2003) [22] found that VC (0.2 % - 0.3 %) was necessary for growth and development of silkworm, while the amount beyond a certain concentration, growth and survival rate are decreased. VC has been reported to inactivate the viruses (Murata & Kato, 1990 [55]; Nagata *et al.*, 2003 [56] and Arakawa, 2007) [8].
VC is present in all plants particularly in fruits and vegetables, representing about 10 % of total carbohydrate pool in favorable conditions (Haytowitz, 1991 [36], Noctor and Foyer, 1998 [58], Smirnoff and Wheeler, 2000 [67] & Smirnoff *et al*. 2001) [68]. VC is also present in mulberry leaves (Lombardi, 1964 [49] and Kanafi *et al.*, 2007) [44]. It has VC content of 1.3 to 1.8 mg/g (Gamo and Nishiyama, 1953) [34]. There is very close relationship reported between the addition of VC in diet and silkworm's growth (Table 1). The growth of 1 gram fresh weight of larva needs about 0.668 mg (0.067%) VC. Although the function of VC to silkworm physiological activities has not been clear, it is known to promote silkworm food ingestion (Junliang *et al.*, 1994). But, the content of VC in mulberry leaves is largely dependent on climatic and seasonal conditions (with a maximum content in late springtime, or at the beginning of summer under temperate climatic conditions) and on the position of the leaf on the shoot (with the maximum content in the apex and median leaves and minimum content near the branch insertion). Furthermore, the choice of the mulberry variety affects the content of VC in the mulberry leaves. VC has been found to be reduced in the mulberry leaves grown under shade. Leaf preservation, even if it is protected from direct sunlight, is responsible for reducing VC content at a rate of at least 20 % in 24 hours (Cappellozza *et al.*, 2005) [15]. Drying of mulberry leaves causes a complete degradation of VC, so mulberry leaves cannot be considered as a source of VC for larvae fed on artificial diet containing even a high percentage of dried, pulverized mulberry leaves. Babu *et al.*, (1992) [10] measured the VC content in M5 mulberry leaf of different ages, fresh and preserved leaves and found that it varied according to the age and preservation of leaf. Gamo (1941) [33] showed that the content of the VC in fresh mulberry leaves changed in relation to conditions influencing the food value of leaves and that the amount of VC in the larval body is dependent on the leaves.
In view of the above conditions which affect the VC content of mulberry leaf, inability of silkworm to synthesize it *de novo*, it becomes inevitable to fortify the mulberry leaves with VC for normal growth and development, enhancement in qualitative and quantitative production of silk.

Table 1: Relationship between the vitamin C in diet and silkworm growth/larva

VC mg/g dry diet	Average body wt. (mg)	
	After 15days	After 25 days
0	36.7	All died
0.2	89.3	All died
0.4	89.4	349
0.8	98.8	586
1.5	96.1	575
3	89.7	922
5	107.8	901
10	97.7	1047
20	96.9	1072
50	56.5	719

Source: Junliang *et al.*, 1994

1.6 Fortification with vitamin C
In higher animals VC supplementation in the diet of tropical fish has been reported to increase stress resistance, effective response of the immune system and normal development (Waagbo, 1994) [72]. Birds require additional supply of VC under heat stress or in case of disease infection (Schmeling & Nockels, 1978 [65] and Kafri & Cherry, 1984) [43]. Dietary supplementation with VC improved growth rate (Pardue & Thaxton, 1986) [60] and feed efficiency (Mohammed, 1995) [52] of broiler chicks. Several investigators have reported improvements in growth (Cromwell *et al.*, 1970 [21]; Yen and Pond, 1981) [73] and meat quality (Mourot *et al.*, 1990 [54]; Kremer *et al.*, 1999) when supplementing swine with VC. Effects of VC on pork quality may be the result of altered glucose and glycogen metabolism (Mourot *et al.*, 1990) [54]. Mahan *et al.* (1966) [50] reported an increased growth rate of pigs when VC was administered orally or injected.
In silkworm, fortification of mulberry leaves fed to silkworm has been reported to increase the food consumption, coefficient of utilization (Javed and Gondal, 2002) [41], larval weight (Etebari *et al.*, 2004) [30], silk filament length and weight (Babu *et al.* (1992) [10] and fecundity (Rahman *et al.*, 1990 [41] & Chauhan and Singh, 1992) [17]. Further, it has been reported that excessive amounts of VC supplementation to silkworm diet have negative impact causing decrease in food intake and cocoon characteristics due to hypervitaminosis (Tantray *et al.*, 2011) [11]. Dietary supplementation with synthetic and plant based VC has been found to enhance the economic parameters of the mulberry silkworm significantly (Tantray *et al.*, 2015, Tantray and Trivedy 2008 & 2011, Tantray, 2017a and Tantray, 2017b).

1.7 Concluding Remarks
This review summarizes data with regard to the various characteristic properties, functions and effects of vitamin C with particular reference to its importance and requirement in insect life visa vis., silkworm. Importance, occurrence and abundance of this master nutrient in plant kingdom and its effectiveness at lower concentrations indicate the need for elaborating comprehensive studies on the effect of botanical based VC on silkworm. It is advisable that we keep in mind the negative effects of enrichment beyond the optimum level beside its positive effects on economic traits and biological parameters.

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