

# **Is the wildlife deficient in the “spark of life”?**

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Thiamine known as vitamin B1 has been attributed with the phrase spark of life, since it is a very important component of neurotransmission, energy and protein production. In the early 20<sup>th</sup> century, epidemic bursts throughout the world were attributed to beri beri, a fatal disease where later was discovered it was due to deficiency in thiamine. The main reason behind thiamine deficiency is malnutrition, since thiamine is only produced by plants and microbes. Since then extensive research on the role of thiamine, thiamine deficiency and treatment in humans has been conducted. However, little are known for the wildlife. Many studies the last 30 years has shown that there is an increasing number of thiamine deficiency incidents in the wildlife in different levels of the food chain. Thiamine deficiency has been documented in many species, among fish, birds, and reptiles. The most devastating consequence of thiamine deficiency is the paralytic idiopathic disorder, which leads to gradual paralysis and death in birds. However, the reason behind thiamine deficiency in wildlife is unknown.

Thiamine is only obtained through diet, with basic source in the food chain being the plants; monitoring of the thiamine levels in plants will give the insight in the thiamine resources getting into the food web. For that it is necessary to develop an analytical method to measure the thiamine levels in plants and leaves. The current project aimed in developing a method which could measure accurately and consistently the thiamine levels in leaves.

The starting point of the method was an analytical method extensively used for animal species. The basic analytical tool of the method is a very well established, called High-Performance-Liquid-Chromatography (HPLC). HPLC is an analytical method where sample, with regulated pressure and flow, passes through an HPLC column, where the target molecules are separated. The different target molecules interact differently with the column and their passing through is delayed. In the end of the column there is a detector, which records the intensity and the different time the target molecules passed through. These information can help us identify the different molecules and the quantity of the molecules in the sample. In the case of this project the target molecules were thiamine, thiamine monophosphate which is the inactive form of thiamine in the cell, and thiamine diphosphate which is the very active form of thiamine in the cell.

The method is divided in two parts, the sample preparation and the HPLC analysis. During the method development there were problems in the sample preparation which led to significant problems with the HPLC analysis. The main issue was impurities that remained in the sample despite the extensive washing steps and treatments. This obstacle was surpassed by addition of an extra filtration step known as Solid Phase Extraction (SPE) process, where non-specific impurities were trapped in cartridges and pure sample was obtained.

This method will be a very useful tool in monitoring thiamine levels in leaf samples and eventually contribute in solving the riddle of thiamine deficiency in wildlife.