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# Is the titer of adipokinetic peptides in *Leptinotarsa decemlineata* fed on genetically modified potatoes increased by oxidative stress?

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ABSTRACT

The level of adipokinetic hormones (AKHs) (Peram-CAH-I and II) in the corpora cardiaca and the hemolymph of *Leptinotarsa decemlineata* enormously increases in the adults fed on genetically modified potatoes containing either GNA lectin or Cry 3Aa toxin concomitant with increased oxidative stress in gut tissues. A similar enhancement of the AKH titer is achieved when the adults are injected with paraquat that evokes oxidative stress. On the other hand, an injection of exogenous AKH reduces oxidative stress biomarkers in the hemolymph by reducing protein carbonyls and enhancing reduced glutathione levels. These facts indicate that there is a feedback regulation between an oxidative stressor action and the level of AKH in the insect body, and that AKHs might be involved in the activation of an antioxidant protection mechanism. These results are to our knowledge, the first evidence for the involvement of AKHs in oxidative stress mitigation, in addition to a plethora of other roles.



## 1. Introduction

Adipokinetic hormones (AKHs) belong to a group of arthropod neuropeptides that increase the concentration of energy substrates in the hemolymph, inhibit synthetic reactions and control a number of corresponding biochemical and physiological actions on the cellular, tissue and organismal levels [33]. The AKHs of insects are usually synthesized and stored in the neurosecretory cells of the corpora cardiaca (CC), a pair of neuroendocrine glands connected with the brain. Dozens of AKHs have been isolated, characterized and synthesized in almost all insect orders [5,6]. Two adipokinetic peptides were also isolated from *Leptinotarsa decemlineata* CC and since they are identical with the AKHs of *Periplaneta americana*, they are referred to as Peram-CAH-I (pGlu-Val-Asn-Phe-Ser-Pro-Asn-Trp-$NH_2$) and-II (pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-$NH_2$) [7]. AKHs generally operate as typical stress hormones that activate reserves to combat stressful situations that insects encounter during their life. Recently we have proven that the level of AKHs also increases in the insect body in stress situations that are not apparently connected with a direct mobilization of energy, e.g. after an insecticide treatment, a photophase interruption [16], and during constant darkness [18].

It is generally accepted that feeding on plants harboring pro-oxidant allelochemicals elicits generation of free radicals

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in the insect gut and causes oxidative stress [30]. Herbivorous insects harbor a suite of antioxidant enzyme systems to combat the deleterious flux of oxidative radicals generated by the oxidation of allelochemicals [1] but specific roles of individual components of these systems have only been partly elucidated. Johnson and Felton [14] found that elevation of foliar phenolics in a genetically modified (GM) tobacco increased antioxidant conditions in insect herbivores. This finding accentuated the need to examine general impact of transgenic products on the plant-insect interaction including the level of plant components that generate oxidative radicals. A proteomic approach to insect resistance to the Cry toxins of *Bacillus thuringiensis* revealed an increase of glutathione utilization and an elevation in oxidative metabolism in the midgut of the Indian meal moth, *Plodia interpunctella* [3]. The authors hypothesized that the increased oxidative metabolism may be an adaptive response mediating detoxification mechanisms in insects exposed to a survival challenge. The titers of reactive oxygen species (ROS) and the activities of antioxidant enzymes in the midgut of *L. decemlineata* fed on potato leaves was recently elucidated by Krishnan et al. [21]. A lower level of ROS and a higher antioxidant potential in the adult than in the larval midgut indicated stage-specificity in the management of oxidative stress. However, there is a paucity of information about possible hormonal mechanisms controlling the activity of antioxidant systems in the insect body. There are indications that glucagon plays a regulatory role in activation of antioxidant mechanisms in vertebrates [24]. Lu et al. [25] reported that glucagon mediated signal transduction pathways lead to a down-regulation of hepatic reduced glutathione (GSH) synthesis while promoting the efflux of GSH to the blood plasma. As AKHs in the insect body play a similar role to glucagon in vertebrates, we decided to test the hypothesis (1) if insect AKHs are affected by stress associated with feeding on GM food, (2) if these peptides could be involved in the activation of an antioxidant protection mechanism, and (3) if both mechanisms are somehow connected.

To address these questions and test the hypothesis, the present study was directed towards examining the effect of genetically modified food expressing *Galanthus nivalis* agglutinin or *Bacillus thuringiensis* toxin on the changes in the titer of AKHs in corpora cardiaca and hemolymph of *L. decemlineata*. The effect of this stressor was compared with another stressing factor i.e. injection of the herbicide paraquat that elicits oxidative stress [12]. Possible similarity of the mechanisms of their action is discussed.

## 2. Material and methods

### 2.1. Insect and food plants

A stock culture of the Colorado potato beetle *L. decemlineata* (Say) selected from wild populations collected in the vicinity of České Budějovice (South Bohemia, Czech Republic, 49°N), was used in this study. Insects (from egg to adult) were kept in a greenhouse at constant temperature of 25 ± 1 °C and under long-day conditions (LD 18:6 h). They were fed on the leaves of the potato cultivar Desireé. In some experiments, freshly emerged adults were moved to the genetically modified potato GNA2#28 expressing GNA (*Galanthus nivalis* agglutinin) lectin or NewLeaf Superior NLS expressing Cry 3Aa (*Bacillus thuringiensis*) toxin. The cultivars were maintained in vitro in the Potato Research Institute, Havlíčkův Brod, Czech Republic.

### 2.2. Extraction of AKH from CC and hemolymph

Methanolic extract (80% methanol) of the corpora cardiaca (CC) was used to determine AKH content by means of a competitive ELISA (see below). For determination of the endogenous AKH titer in the hemolymph by the ELISA, some pre-purification steps as described elsewhere [8] with some modifications, were essential. Hemolymph samples, always collected from 8–10 individuals were extracted in 80% methanol and after centrifugation the supernatants of these extracts were evaporated to dryness. Solid phase extraction (Sep Pak C18, Waters) and HPLC pre-purification were then employed. Briefly, fractions eluting in 60% acetonitrile from the Sep Pak column were evaporated again and taken for HPLC analysis on the Waters chromatography system, at a flow rate 2 ml/min and fluorometric detection at $\lambda_{Ex}$ = 280 nm and $\lambda_{Em}$ = 348 nm. The samples were fractionated on the Chromolith Performance RP-18e column (Merck) 100 × 4.6 mm with gradient 0–2 min 25% B, 2-12 min 25–87% B and 12-15 min 87% B (A = 0.11% trifluoroacetic acid (TFA) in water, B = 60% acetonitrile in 0.1% TFA). Fractions eluted between 6.1–8.1 min were then taken for the ELISA competitive tests (retention times of the synthetic Peram-CAH-I and Peram-CAH-II were 6.5 and 7.5 min, respectively, in this system, Fig. 1). The efficiency of recovery of hemolymph AKH during the extraction procedure was checked by adding 500 fmol of Pyrap-AKH (see below) to 20 μl samples of hemolymph before the extraction. The recovery of AKH (74.8 ± 8.2%; mean ± S.E.) was checked using ELISA (see below) and estimated from five separate parallel measurements; all respective data were corrected for the corresponding losses.

### 2.3. Quantification of AKH by ELISA

A competitive ELISA was used for determination of the AKH content in the CC (1/4 CC equiv./well) and the hemolymph (10 μl equiv./well) according to our protocol described earlier

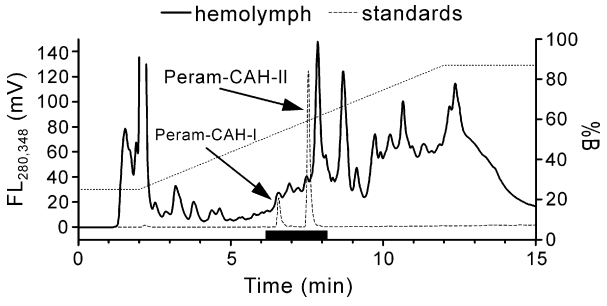


**Fig. 1 – An example of RP HPLC profiles (fluorometric detection at $\lambda_{Ex}$ = 280 nm and $\lambda_{Em}$ = 348 nm) of an extract from 40 μl pre-purified hemolymph from 1-day-old *L. decemlineata* and the Peram-CAH-I and II standards (ca. 100 and 150 pmol). The solid bar represents the fraction taken for ELISA. For details see Section 2.2.**