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CAMPUS DE BOTUCATU

SUPLEMENTAÇÃO DE FONTE INORGÂNICA DE ZINCO NO PERFIL PROTEÔMICO E METALÔMICO DA GELEIA REAL EM ABELHAS *Apis mellifera* **L.**

AIMÊ DE ALMEIDA LONGUINI

Dissertação apresentada ao Programa de Pós-graduação em Zootecnia como parte das exigências para obtenção do título de Mestre em Zootecnia

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> “Mas tu não deves esquecer. Tu te tornas eternamente responsável por aquilo que cativas.”

O pequeno príncipe - Antoine de Saint-Exupéry

RESUMO GERAL

Este estudo teve como objetivo avaliar a qualidade nutricional do perfil proteico da geleia real produzida pelas abelhas *Apis mellifera* suplementadas com diferentes concentrações de zinco inorgânico (Sulfato de Zinco monoidratado - 0, 25, 50 e 75 ppm). Foi feita eletroforese bidimensional para o fracionamento das proteínas da geleia real e o nível de zinco quantificado pela técnica de espectrometria de absorção atômica com chama (FAAS). A análise de proteínas da geléia real como componente extracelular foi realizada pela plataforma Blast2Go, onde foram obtidos gráficos de funções moleculares e processos biológicos das proteínas encontradas através da análise proteômica por análise de espectrometria de massa ESI MS / MS, mostrando as proteínas "Major Royal Jelly Protein 1" e "Major Royal Jelly Protein 8" como as principais proteínas ativas na geléia real tanto para up como para down regulation e associada ao zinco. Todos os tratamentos independentes das concentrações de zinco mostraram número menor de *Spots* proteicos quando comparadas com o controle. Todas as proteínas contendo zinco foram classificadas como Proteínas Principais da Geleia Real (MRJPs). A exposição de abelhas nutrizes ao mineral zinco em sua forma inorgânica promoveu aumento na expressão de proteínas da geleia real envolvidas em sistemas de defesa (MRJP8 e MRJP9), entretanto reduziu a expressão de seis diferentes proteínas das MRJPs (sendo elas: MRJP, MRJP1, MRJP2, MRJP3, MRJP5 e MRJP7). Nossos resultados demonstram que proteínas vitais e processos metabólicos são prejudicados em abelhas nutrizes expostas ao mineral zinco em sua forma inorgânica em todas as doses utilizadas afetando a nutrição e manutenção de colônias.

Palavra - chave: abelhas, mineral, proteômica, suplementação.

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SUPPLEMENTATION WITH AN INORGANIC ZINC SOURCE IN THE METALLOPROTEOMIC PROFILE OF ROYAL JELLY *IN Apis mellifera* L.

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ABSTRACT

This study aimed to evaluate the quality of royal jelly produced by honey bees *Apis mellifera* supplemented with different concentrations of inorganic zinc (Zinc Sulphate monohydrate - 0, 25, 50 and 75 ppm). Was performed two-dimensional electrophoresis for the fractionation of royal jelly proteins, and the zinc level was quantified by the flame atomic absorption spectrometry (FAAS) technique. Proteins were identified by electrospray ionization mass spectrometry (ESI MS MS). Analysis of variance followed by the Tukey test ($P < 0.05$) was used. Supplementation with the mineral zinc positively affected the quantification of proteins for treatments 50 and 75 ppm. However, all treatments independent of zinc concentrations showed fewer protein spots when compared to the control. All zinc-containing proteins were classified as Major Royal Jelly Proteins (MRJPs). The exposure of nursing bees to the mineral zinc in its inorganic form reduced the expression of six different MRJP proteins involved in larval development and expression in the feeding glands of nursing bees (MRJP1, MRJP2, MRJP3, MRJP5 and MRJP7), however promoted an increase in the expression of royal jelly proteins involved in defense systems (MRJP8 and MRJP9). The results demonstrate that vital proteins and metabolic processes are impaired in nursing bees exposed to the mineral zinc in its inorganic form in all doses used affecting nutrition and maintenance of colonies.

Keywords*:* bees, mineral, proteomic, supplementation.

INTRODUCTION

In order for the honey bee colony *Apis mellifera* to express its full productive and reproductive potential, specific nutrients are essential to provide maintenance and survival [1, 2]. Among the main foods consumed by honey bees, royal jelly, formed from the mixture of secretions from the hypopharyngeal glands (clear secretion rich in proteins) and mandibular (milky white component) [3, 4, 5, 6].

Royal jelly is extremely important for the colony, serving as food for worker larvae, queens and drones until the third day of life; after this period, worker larvae and drones receive pollen and nectar / honey as the main food and small amounts of royal jelly; however, the queen larva receives exclusively royal jelly throughout its larval development and adult life, promoting physiological changes such as the

development of structures related to reproduction [4, 7, 8]. For adequate production of royal jelly by young worker bees, they need adequate amounts of protein in their diet [3]. The composition of royal jelly can also vary depending on the age of the larva, environmental conditions, physiological and metabolic state of the nursing bee and pheromones in inducing the production of royal jelly by the hypopharyngeal and mandibular glands [9]. Royal jelly has water-soluble proteins in its composition (Water Soluble Proteins - WSP) that have outstanding nutritional value and are called Major Jelly Proteins or Major Royal Jelly Proteins (MRJPs), where MRJP1, MRJP3 and MRJP5 are responsible for 82% - 90% of total royal jelly proteins [10, 6], whose relative molecular mass is 49 to 87 kDa [11]. So far, nine MRJP proteins have been found in *Apis mellifera* (MRJP1, MRJP2, MRJP3, MRJP4, MRJP5, MRJP6, MRJP7, MRJP8, MRJP9), and an incomplete polypeptide, MRJPψ, encoded by a pseudogene however, little is known about the functions of each [10].

Mineral sources are obtained through pollen, nectar and water, [12] propose a diet with 1000 ppm potassium, 500 ppm calcium, 300 ppm magnesium and 50 ppm sodium, zinc, manganese, iron and copper. [13] suggest 30 ppm of zinc for survival and anti-oxidant activity for honey bees and 60 to 70 ppm for the production of royal jelly. However, there is little knowledge about the need for these micronutrients and their physiological influences on honey bees [14, 15]. Minerals like zinc are fundamental as structural and functional components; in the structural aspect it is present in metalloproteins, and in the functional aspect, it acts as an enzymatic cofactor, participating in metabolism, regulation of gene expression, structural maintenance of biomembranes, immunity and protection against free radicals, protein synthesis, among others [16, 17]. Protein-bound zinc exerts enzymatic catalytic and inhibitory functions, arrays and breakdowns of macromolecular subunit complexes and protein / receptor complex formation [16, 1].

The areas of science called metallome and metalloproteome allow the assimilation between analytical studies and inorganic and biochemical studies [18], therefore the metalloma can present data as (1) metallic or metalloid species divided between the cell compartments of a certain type cell; (2) which biomolecule is incorporated or which bioligand is complexed; (3) the concentrations of the individual metallic species present and (4) the biomolecule structure [18, 19, 20].

Therefore, in order for there to be a greater understanding of the real nutritional needs of the colonies, evaluations using techniques such as proteomics and metalloproteomics will provide an understanding of how minerals linked to protein act in the organism of honey bees. There are still gaps regarding the correct availability of the mineral.

MATERIAL AND METHODS

- Study Site

The experiment was conducted at the apiary in the Beekeeping Production Area of the Lageado Experimental Farm, Faculty of Veterinary Medicine and Animal Science, UNESP, Botucatu, São Paulo, Brazil. It was conducted at the following geographic coordinates: 22° 49′ south and 48° 24′ west. The place is characterized by a humid subtropical climate and an average altitude of 623 m.

- Experimental Groups

There is little information regarding the correct availability of the mineral zinc in the nutrition of bees and its appropriate level to be applied. However, the composition of royal jelly can be affected according to the available food source. Therefore, metalloproteomics is an important tool in understanding royal jelly, highlighting the proteins associated with the mineral and showing its importance to honey bees.

CONCLUSIONS

It was concluded that the exposure of nursing bee to the inorganic zinc mineral form reduced the expression of six of the Major Royal Jelly Proteins (Down regulation: MRJP, MRJP1, MRJP2, MRJP3, MRJP5 and MRJP7), however it promoted an increase in the expression of jelly proteins involved in defense systems (Up regulation: MRJP8 e MRJP9). Our results demonstrate that vital proteins and metabolic processes are impaired in nursing bees exposed to the mineral zinc in its inorganic form in all doses used affecting nutrition and maintenance of colonies.

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AUTHORS CONTRIBUITION

All authors have contributed equally to the work.

CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest in relation to the study in this paper.

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