

Mercury, lead, arsenic and cadmium in fresh and frozen farmed shrimp and pond water from three shrimp farms

Mercúrio, chumbo, arsênio e cádmio em camarões frescos e congelados coletados em três fazendas

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Trace metals are not obvious pollutants present in shrimp farm effluents. However, some trace metals are present as natural components in aquafeeds, as impurities in fertilizers or as active principles of pesticides, which can be accumulated in shrimp tissue. It were analyzed 90 muscle samples of fresh and frozen farmed shrimp and pond water collected in three farms in Brazil, between September 2008 and March 2011. It were determined total mercury concentrations by a flow injection mercury system and, lead, arsenic and cadmium concentrations by graphite furnace atomic absorption spectrophotometry. Mercury levels in the farmed shrimp ranged from 0.01 – 0.18 mg.kg⁻¹ in fresh shrimp, and from 0.002 – 0.008 mg.kg⁻¹ in frozen shrimp (wet weight). A similar behaviour was observed to lead concentrations, since frozen shrimp presented markedly lower lead concentrations (0.30 – 0.89 mg.kg⁻¹) than fresh shrimp (0.92 – 2.89 mg.kg⁻¹). In the case of arsenic, were observed concentrations of 0.01 – 0.77 mg.kg⁻¹ in fresh shrimp muscle, and were no detected concentrations of this trace metal in muscle of frozen shrimp (Limit of detection of 0.002 mg.kg⁻¹). The shrimp freezing process consists of a preliminary washing thoroughly in chlorinated water (5 ppm) to remove any remaining mud or sand, and to reduce bacterial contamination. The shrimp are drained as much as possible and are then ready for freezing through quick-freezing tunnel (individually quick frozen). There is no evidence indicating that this process contributed to the reduction of trace metals levels in frozen shrimp, but is clear that lead, arsenic and mercury levels are markedly lower in frozen than fresh shrimp. It was observed low concentrations of trace metals in pond water and also in shrimp muscle. In relation to the monitored years, in 2008 it was observed a slightly higher concentration of all trace metals in fresh shrimps compared to other years. Episodes of intensive precipitation caused recurrent devastating floods in the region in 2008, and caused harm to farms evaluated. Thus, increased concentrations of these trace metals in the rainy season may be related to resuspension of particles deposited in the sediment of the bottom in the ponds and therefore available for incorporation into these trace metals by aquatic biota. It can be concluded that farmed shrimp in Brazil is safe for human consumption. Thanks go to CNPq/ MAPA/SDA 577906/2008-9 for financial support and scholarships conceded.

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Total mercury in feed, muscle and liver of farmed Nile tilapia

Mercúrio total em rações, filés e fígado de tilápia do Nilo de pisciculturas

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Fish consumption is considered the most important source of contaminant exposure for humans beings, and farmed fish can be exposed to contaminants

via feed supply. Total mercury concentrations (THg) were determined in the muscle, liver and feed of farmed Nile tilapia (juveniles and adults) from four different fish farms (net cage and pond systems), by a flow injection mercury system. Mercury concentrations observed in muscle and liver were lower than the values recommended by Brazilian regulatory agency (Anvisa) for fish of 500 µgTHg.kg⁻¹ and fish farm NC₁ showed the highest THg concentrations when compared to the other fish farms. There are reports of strong gold exploration around 1987 in this region, where more than 100 ferries worked in mining this metal. The low THg concentrations found in muscle of farmed tilapia in this study confirm the low tendency of these fish to accumulate Hg, since they are less exposed to this contaminant than wild tilapias. Other factors, such as higher growth rate and shorter life span when compared to wild fish can contribute to marked reductions in THg concentrations. Body lipid content can also influence contaminant concentrations accumulated in an organism. Lipid concentrations in the present study in fish muscle ranged from 0.7 to 4.3%, noting that lipid levels were higher in NC than in PS fish farms. Thus, higher THg concentrations in the muscle tissue with lower lipid concentrations (PS farms) were observed. The low THg concentrations found in the analysed fish feed (5.2 – 33.2 µg.kg⁻¹) can be also responsible for the low concentration in fish muscle. Therefore, continuous monitoring of contaminant concentrations in fish feed is necessary due to variations in the use of ingredients, which cause variations in the concentrations of undesirable substances in commercial feeds. In relation to tissue analysis, a statistically significant difference (P < 0.05) between Hg accumulation in liver and muscle was observed, where the liver presented the highest concentrations. Regarding fish age difference, a statistically significant difference (P < 0.05) was observed when comparing Hg accumulation between adults and juveniles, with larger fish having higher THg concentrations than smaller fish. Differences in bioaccumulation rates can be related to the different nutritional needs of fish in different life stages. Thus, fish age is an important factor, due to changes in food supplies, as well as the increased exposure time of the animals to Hg.

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Development of an immunoassay for the differential diagnosis of trypanosomiasis of veterinary interest: A preliminary proteomic survey of *Trypanosoma evansi* trypomastigotes using lc/ms/ms approach for differential diagnostics

*Desenvolvimento de um imunoensaio para diagnóstico diferencial da tripanossomíase de interesse veterinário: Um levantamento preliminar da proteômica de tripomastigotas de *Trypanosoma evansi* usando a abordagem LC/MS/MS para o diagnóstico diferencial*

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The veterinary trypanosomes *Trypanosoma evansi* and *Trypanosoma vivax* are parasites that affect many animals species such as horses, camels and bovines causing significant economic losses to livestock industry around

the world. Recently, human cases have been described. In Brazil, outbreaks of these parasites have been reported in several states from north to south. The differential diagnosis is not efficient and there is no differential field test to be used. Thus using the proteomic approach, we began the study of proteins differentially expressed in each of the parasites. The aim of this study is to identify proteins expressed by *T. evansi* trypomastigotes in mouse experimental infections, with emphasis in the identification of potential drug targets and differential diagnostic antigens. In a preliminary proteomic survey using LC/MS/MS, 33 proteins from *T. evansi* trypomastigotes were identified and assigned to COG functional groups, with most of them belonging to the group of cellular processes and signaling and metabolism proteins. Among the identified proteins there were cyclophilin and cysteine peptidase, which have been described as virulence factors and potential drug targets in other trypanosomatids, for being involved in parasite growth and survival in mammalian hosts. Furthermore, we identified several glycolytic enzymes, such as ATP-dependent phosphofructokinase, enolase and glycosomal fructose-bisphosphate aldolase. In *T. evansi* these enzymes are especially important for flagellar movement, a process that is dependent of the environmental glucose concentration. Considering that flagellar movement is essential for parasite infection, glycolytic enzymes also become attractive targets for future studies on their drug target potential. The *T. evansi* proteomic survey will also be extended with the analysis of more trypomastigote samples by both LC/MS/MS and 2DE-MALDI-MS/MS, in order to provide a comprehensive coverage of the repertoire of proteins expressed by this stage of the parasite. For the identification of antigenic proteins with potential for use in "surra" immunodiagnosis, we will also perform 2DE-immunoblots with sera from animals infected with *T. evansi* trypomastigotes.

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Detecção de polimorfismos do gene da proteína priônica no rebanho ovino nacional: métodos e aplicabilidade à seleção para resistência ao Scrapie*

Detection of polymorphism of the prion protein gene in national sheep flock: method and applied for selection and resistance to Scrapie

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Scrapie ou paraplexia enzoótica dos ovinos é uma doença neurodegenerativa fatal que acomete ovinos e raramente caprinos. O agente causador é chamado de príon (PrP^{Sc}), uma forma alterada da proteína priônica normal (PrP^C). A PrP^C está presente na membrana celular de diversas células dos animais, principalmente no SNC. A infecção pelo PrP^{Sc} pode ocorrer mais comumente por três formas: hereditariedade, consumo de alimentos oriundos de animais portadores da PrP^{Sc} ou por eventuais polimorfismos. Os polimorfismos ocorrem nos códons 136, 154 e 171 do gene PRNP, que é o responsável pela síntese da proteína priônica. Os animais podem ser susceptíveis ou resistentes, de acordo com as

seqüências alélicas observadas nos referidos códons. São considerados altamente susceptíveis ao scrapie, animais que apresentem no seu genótipo a combinação VRQ para o gene PRNP, independente do outro alelo. São considerados como susceptíveis ao scrapie, ovinos que apresentem no seu genótipo a combinação alélica ARQ, AHQ, ou ARH para o gene Prnp. São considerados resistentes ao desenvolvimento de scrapie os ovinos que apresentem no genótipo dois alelos ARR. No Brasil, ocorreram apenas casos de animais que foram importados, sendo o País considerado livre da doença. Neste trabalho, foi realizada a genotipagem dos diferentes polimorfismos associados ao desenvolvimento do Scrapie e a categorização em animais susceptíveis e resistentes. Foram sequenciadas 118 amostras provenientes de ovinos nacionais da raça Santa Inês. Dessas amostras, foram identificados seis alelos e 11 genótipos (ARQ/ARQ, ARR/ARQ, ARQ/AHQ, ARQ/VRQ, AHQ/AHQ, ARR/ARR, ARR/AHQ, VRQ/VRQ, ARQ/TRQ, TRR/TRR, TRQ/TRQ), dentre os quais o genótipo ARQ/ARQ teve prevalência de 56,7%. Apenas 1,69% das amostras analisadas possuem o genótipo ARR/ARR, considerado resistente à doença. Em nove amostras, pôde ser observada a presença da Tirosina no códon 136, culminando com os seguintes genótipos: A/T e T/T. Até o presente momento, esses polimorfismos só foram descritos em publicações internacionais e são considerados como observações raras. Esse é o primeiro relato nacional e o primeiro relato envolvendo a raça Santa Inês. O efeito na susceptibilidade ou resistência nos animais portadores da homozigose T/T no códon 136 ainda não está totalmente elucidada, já a heterozigose A/T não altera a susceptibilidade à doença. Esses resultados apresentam grande variabilidade genética relacionada à raça Santa Inês e a baixa frequência do alelo ARR no rebanho nacional demonstra que programas de melhoramento genético envolvendo a raça Santa Inês devem ser adotados para aumentar a resistência ao desenvolvimento da doença.

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Determinação de microorganismos responsáveis pela deterioração em carnes refrigeradas embaladas a vácuo*

Determination of microorganisms responsible for deterioration in vacuum packed chilled meat

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O estufamento de embalagens a vácuo de carnes refrigeradas, também conhecido como *blown pack*, é atribuído a bactérias psicrófilas e psicrotólicas, dentre as quais fazem parte algumas espécies de clostrídios e enterobactérias. Essa deterioração é caracterizada pela intensa distensão da embalagem, levando à produção de gases e odores desagradáveis. O objetivo deste trabalho