Concentrations of carnosine, anserine, L-histidine and 3-methyl histidine in boar spermatozoa and sheep milk by a modified HPLC method

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Abstract

The present study deals with the application of high-performance-liquid-chromatography (HPLC) method for a quantitative detection of carnosine, anserine, L-histidine and 3-methyl-L-histidine in biological material with o-phthaldialdehyde (OPA) post column derivatisation at the constant temperature of 50°C. For this purpose, some mobile-phases were prepared with scalar acetonitrile concentrations. A complete separation of all molecules, particularly for carnosine and 3-methyl-L-histidine, was obtained with a solution of acetonitrile and 6mM hydrochloric acid with 0.48 M sodium chloride (5%:95% v/v). Post column derivatisation reaction at temperature of 50°C permitted to obtain an increase in sensibility of all molecules. This method has been utilised for detection of histidine dipeptides in boar spermatozoa and in sheep milk. Concentrations (mean ± S.E. nmol/10⁹ spermatozoa) of carnosine (0.96 ± 0.14) and anserine (0.83 ± 0.18) in boar spermatozoa were significantly lower than those of L-histidine (52.85 ± 4.86) and 3-methyl-L-histidine (83.07 ± 7.1). Positive correlation was found between carnosine and anserine contents (r=0.740; p<0.01) and between L-histidine and 3-methyl-L-histidine (r=0.657; p<0.01). All histidine dipeptides studied were also present in 40 samples of sheep milk. In a case of samples without unit-forming colonies (UFC) of Staphylococcus coagulase-positive, carnosine concentrations (9.17 ± 0.89 nmol/ml) were higher than anserine (0.51 ± 0.02 nmol/ml) and both were significantly lower in respect to L-histidine (49.51 ± 6.48 nmol/ml) and 3-methyl-L-histidine (81.21 ± 6.82 nmol/ml). A negative correlation was observed between carnosine milk levels (r=-0.773; p<0.01) and UFC/ml of Staphylococcus coagulase-positive. In conclusion this very simple and fast method can be used to detect histidine dipeptides in biological compartments where their concentrations are very low.

Key words: HPLC method, histidine dipeptides, boar semen, sheep milk.