

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 \pm 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.