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PH INFLUENCE ON DIFFERENT BOVINE TESTICULAR HYALURONIDASE DETERMINATION ASSAYS

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Abstract. Hyaluronidases from testes are enzymes that degrade hyaluronic acid and are essential for fertilization. A soluble fragment from the bovine testicular hyaluronidase PH-20 (BTH), was extensively characterized. However, many studies in literature show variable data regarding the optimum pH conditions for BTH enzymatic activity. The objective of this study was to evaluate the optimum pH in different conditions in parallel assays. The methods employed here were based on the colorimetric technique that determines the amount of N-acetyl-D-glucosamine released after the enzymatic reaction and on zymography that evaluates the degradation of the substrate copolymerized within the polyacrylamide gel matrix. Our results show surprising differential behavior of the enzyme under the abovementioned different assays.

Key words: BTH, enzyme activity, pH**,** zymography.

Introduction

Hyaluronidases were first identified in extracts from mammalian testes. These enzymatic activities were demonstrated to be of type endo-*N*-acetylhexosaminidases that hydrolyse hyaluronan (HA), a glycosaminoglycan of a several million of Da, to tetrasaccharides as the main end product [1,2].

Testicular hyaluronidases (PH-20) are membrane-bound proteins that present a glycosyl-phosphatidyl-inositol (GPI) anchor that allows them little freedom, keeping them associated with the cell surface. This enzyme was demonstrated to have an optimum pH in the neutral range owing to the fact that it should be active in physiological conditions in order to perform the fecundation [3].