2001) was used as tracer and antigen for antiserum production in rabbits. The purified zebu PAG-1 was radiolabelled by lactoperoxidase method. Unreacted iodine was discarded by gel filtration. The immunizing dose for antiserum production was 250 µg dissolved in 0.01 M phosphate buffer, pH 7.4. Dilution curves determined the optimal dilution of the antiserum (binding of 20–30% of tracer with nonspecific binding 2%). In the presence of antibody in excess, 73.6% of labeled zebu PAG-1 was bound. The optimal dilution of the antiserum issued from the first bleeding (1-week after the third injection of antigen) was 1:100,000. These first results indicate that a new homologous radioimmunoassay system is now available for physiological investigations on secretory profiles of PAG in zebu cattle.

Further investigations are in progress to produce new tracers and antisera for the development of a highly sensitive radioimmunoassay method for PAG detection in peripheral blood of pregnant females. This new system could be used in experimental and/or farm conditions in order to improve the knowledge about the endocrine physiology of zebu females.

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RADIOIMMUNOASSAY OF PORCINE PEPSINOGEN

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Keywords. Pepsinogen, radioimmunoassay, sensitivity, specificity, swine.

Pepsinogens are the proenzymes of pepsins, they belong to the group of gastric acid aspartic endoproteinases. Exocrine secreted pepsinogens function as zymogens for the main proteolytic activity of gastric juice. There is also a small secretion in blood, the function of this process is not understood. However, exploiting this endocrine process, we can get information on the condition of the gastric mucosa by measurement of the plasmatic or seric concentration of pepsinogen (Biemond *et al.*, 1989). In this abstract we describe the development of a RIA of porcine pepsinogen. Using the Vaitukaitis (1971) method, antisera were raised in rabbit immunized with commercial porcine pepsinogen or with the same preparation treated by gossypol. The purpose of this treatment was to avoid the transformation of pepsinogen into pepsin (cleavage of the 41 amino acid residues from the NH_2 terminus) according to the method of Wong *et al.* (1972).

Tracer was prepared using ^{125}Iodine and two methods: the lactoperoxydase and chloramine T. Buffer for incubation was prepared with Tris-HCl (0.01 M, pH 7.5), BSA 0.1% additionned with Tween 20 0.5% v/v and an inhibitor of aspartic protease: the pepstatin 5 mg/L. Incubation was realized during 24 or 48 hours at 4°C. The separation of free and bound fractions was completed by using a solution containing an anti immunoglobulin antiserum, normal rabbit serum, and polyethylenglycol. The reaction was allowed for 30 minutes. After aspiration of the supernatant and washing of the pellet, the tubes were counted in a Gamma counter (Wallac LKB, Finland).

Rabbits immunized with native pepsinogen gave antisera with high titres, whereas the rabbits immunized after treatment of pepsinogen by gossypol gave lower titre in dilution test.

The lactoperoxydase method gave high non-specific binding and low incorporation of ^{125}Iodine. The chloramine T method produced a tracer allowing a highly sensitive RIA with very low non-specific binding and a high specific binding. The standard curve ranged from 0.2 ng to 100 ng/mL (**Figure 1**). There was no cross-reaction with others members of aspartic proteinases such as renin, cathepsin D and bovine PAG.

Preliminary comparison showed that the Tween and the pepstatin in buffer decrease the NSB and increase the sensitivity of RIA system. Our data show that the RIA is available for determination of pepsinogen circulating in blood and suggest that addition of Tween and pepstatin in buffer could improve the performance of the RIA of other members of proteineases aspartic family.

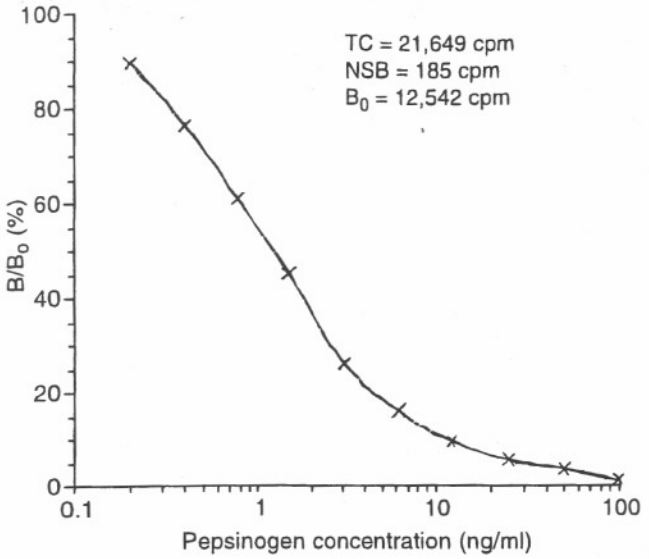

Figure 1. Standard curve for the radioimmunoassay of porcine pepsinogen.

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THE INACTIVE MEMBERS OF THE ASPARTIC PROTEINASE FAMILY IN THE RUMINANT PLACENTA: SPECIFICITY OF THREE DIFFERENT RADIOIMMUNOASSAY SYSTEMS

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Keywords. Aspartic proteinase, radioimmunoassay, specificity.

Pregnancy-associated glycoproteins (PAGs) have been isolated from the placenta of various ruminant species in the recent decade. Molecular biology studies showed that these glycoproteins are inactive members of the aspartic proteinase family (Xie *et al.*, 1991). Radioimmunoassay developped to detect PAGs in biological fluids (Zoli *et al.*, 1992) became important tools for establishment of pregnancy diagnosis and pregnancy follow-up. As the PAGs share a high sequence homology with each other and with the other members of the aspartic proteinase family: cathepsin D, E chymosin pepsinogen and renin, in the present study the specificity of three commonly used RIA systems was tested.

In the three RIA systems 67 kDa PAG preparation was used as tracer (labelled with ^{125}Iodine according to the lactoperoxidase method) and as standard. In RIA 1, the antiserum was raised against 67 kDa PAG purified from bovine placenta. In RIA 2 and 3, antisera contained antibodies against cPAG 55+62 and cPAG 55+59 previously isolated from caprine placenta (Garbayo *et al.*, 1998). Serial dilutions ranging from 10 ng/ml to 1 mg/ml prepared from pepsin, pepsinogen, rennin and rennet in Tween Tris buffer were tested in the three systems in comparison with the PAG standard used for assays.

There was weak inhibition of binding caused by the four preparations examined in the concentration range of 10 ng/ml – 100 mg/ml. Pepsinogen caused a mild inhibition of binding in RIA 2 system at 500 mg/ml (B/B_0=92.81%) and 1 mg/ml (B/B_0= 90.07%) concentrations. In the case of pepsin slightly

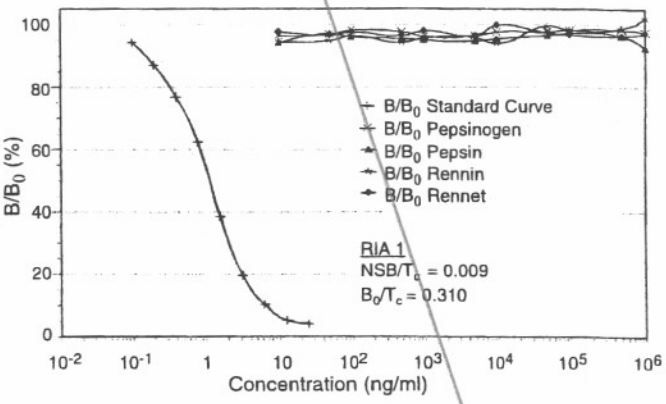

Figure 1. RIA 1: inhibition of binding by pepsinogen, pepsin, rennin and rennet.

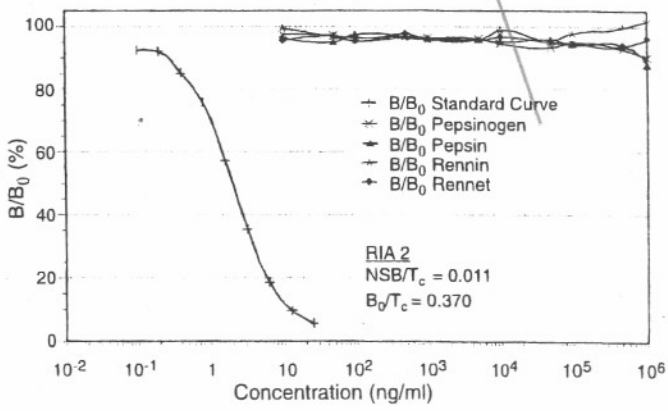

Figure 2. RIA 2: inhibition of binding by pepsinogen, pepsin, rennin and rennet.