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PORCINE HAPTOGLOBIN PURIFICATION THROUGH GEL FILTRATION

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Introduction: Haptoglobin (Hp) is an α2-glycoprotein which participes in haemoglobin transport. It is one of the moderated acute phase proteins in swine, showing increases during various natural and experimental induced infections, and it has been proposed as a tool for health status monitoring in pig production system. In this report we describe a simple protocol for porcine haptoglobin purification based on gel filtration by using a FPLC system in order to improve the speed and decrease the complexity of current procedures for haptoglobin purification.

Material and Methods: Serum with high Hp concentration was obtained from 3 month old pigs with acute rectal prolapse. Specimens were collected by yugular venipuncture and serum was pooled and stored at –20ºC. Three ml of the serum rich in Hp were saturated by 50% ammonium sulphate. After a gentle stirring for 30 min at room temperature, the precipitate was discarded by centrifugation and the supernatant was dialyzed against NaCl 0.9% by using NAPTM 10 desalting columns (Amersham Biosciences). After dialysis, the solution was concentrated by Centriconâ (Amicon, Millipore, USA) and followed by an adition of ultrapure urea to 6M and then filtered through a 0.22µm pore-size filter (Millexâ-GV). The final solution was applied to a SuperdexTM 200 10/300 GL column (Amersham Biosciences, Uppsala, Sweden), equilibrated and run with TSA buffer, pH 7.5 with a flow rate of 20ml/h at room temperature. Fractions were collected using a fraction collector (Amersham Biosciences). The purity of the Hp preparation obtained was assessed by SDS-PAGE using a 5% stacking gel and 12% resolving gel of acrylamide.

Results: The chromatogram obtained by FPLC showed 3 peaks, the second one corresponded mainly to haptoglobin as it could be seen by SDS-PAGE. The content of this peak yielded two bands, the first one with a molecular size of 44 kDa, corresponding to the heavy chain of Hp (b), and the second one with 12.8 kDa, corresponding to the light chain of Hp (a). Minor impurities of 24 and 11.20 kDa were also observed.

Discussion: Molecular size of the protein purified in this study agrees with previous reports and therefore the procedure describe here could be widely used for porcine Hp purification. The present work could provide a more simplified way of purifying Hp than the ones described by Shim et al., (1971) and Hiss et al., (2003), since we only use a chromatographic step and might be an alternative to the method described by Yang & Mao (1999), also based on an unique chromatographic step, in laboratories in which FPLC were available. The purified protein could be used for improvement of current Hp assays or for the development of new and highly sensitive assays such as time-resolved fluoroimmunoassays, in which pure Hp is needed for use as primary standard.

References:

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